PROJECT REVIEW

"*Direct gene doping detection: generation, characterisation and validation of a synthetic reference material for routine testing*"

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The anti-doping community is focusing efforts on developing methods to detect gene doping, a new threat to the world of sport.

Methodology with potential to detect 'doping' genes in blood samples from athletes has recently been developed by us and other researchers. It involves PCR assays targeting intronless cDNA sequences that are present in doping genes but absent in intron-containing endogenous genes. Acceptance of this methodology for routine gene doping testing requires reference materials as controls to ensure the method performs as intended. A positive control containing the cDNA sequence for the candidate doping gene is commonly used in method development. Inadvertent cross-contamination of a tested sample with this type of control would lead to a false positive test result. Hence, it is not suitable for routine testing because of legal implications.

We propose to develop a DNA reference material for a positive control in gene doping testing that will overcome this problem. Using erythropoietin as a model doping gene, we will produce a reference material that will be detectable by the PCR assays with similar specificity and sensitivity as the doping gene. However, the reference material and gene doping products will differ, allowing easy discrimination between true positive and false positive test results. We will characterise this reference material for purity and quantity using latest technologies including digital PCR and next generation sequencing. In addition, using this reference material we will in vitro validate the complete erythropoietin gene doping detection method from sample processing to PCR detection.

This model system will serve as a prototype for preparing reference materials for detecting doping with other candidate genes.

The proposed research is crucial in the development of a routine method for direct detection of gene doping and for ensuring the method is reliable, reproducible and robust and will withstand legal scrutiny.

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Results and Conclusion

The anti-doping community is focusing efforts on developing methods to detect gene doping, a new threat to the world of sport. Recently, methodology with potential to detect 'doping' genes in blood samples from athletes has been developed by us and other researchers.

Acceptance and implementation of this methodology for routine gene doping testing requires Reference Material (RM) as a control to ensure the method performs as intended. Existing 'in-house' controls based on complementary DNA (cDNA) for targeted genes will not withstand legal scrutiny since any cross-contamination will generate 'false positive' test results.

In this project, we generated and characterised a DNA RM that will overcome this problem. Using EPO as a model doping gene, we produced three forms of the RM. Each form is detectable by the PCR assays that target a doping EPO gene. However, the products generated in these assays from the RM and a doping gene differ, allowing easy discrimination between true positive and 'false positive' test results. We characterised these three forms of the RM for purity, quantity and stability, and studied their performance in gene doping detection assays. Finally, the three forms were in vitro validated in the complete EPO gene doping detection method from sample processing to PCR detection.

This model system will serve as a prototype for preparing RM for detecting doping with other candidate genes. The produced RM is crucial in the development and acceptance of a routine test for direct detection of gene doping and for ensuring the method is reliable, reproducible and robust and will withstand legal scrutiny.